

CLAIMS

1. A recombinant marker gene encoding an orotate transporter polypeptide comprising an amino acid sequence at least 60% identical to SEQ ID NO: 2.

2. The marker gene of claim 1, which is a selection marker, a screening marker, a counter-selection marker, and/or a bi-directional selection marker.

3. The marker gene of claim 1 or 2, wherein the encoded orotate transporter polypeptide also transports one or more orotate analogues.

4. The marker gene of any of claims 1-3, wherein the encoded orotate transporter polypeptide also transports the orotate analogue 5-fluoroorotate (FOA).

5. The marker gene of any of claims 1-4, which is transcribed from at least one heterologous and/or artificial promoter.

6. A polynucleotide construct comprising at least one recombinant marker gene encoding an orotate transporter polypeptide comprising an amino acid sequence at least 60% identical to SEQ ID NO: 2.

7. The polynucleotide construct of claim 6, wherein the at least one recombinant marker gene is a selection marker, a screening marker, a counter-selection marker, or a bi-directional selection marker.

8. The polynucleotide construct of claim 6 or 7, wherein the encoded orotate transporter polypeptide also transports one or more orotate analogues.

9. The polynucleotide construct of any of claims 6-8, wherein the encoded orotate transporter polypeptide also transports the orotate analogue 5-fluoroorotate (FOA).

10. The polynucleotide construct of any of claims 6-9, wherein the marker gene is transcribed from at least one heterologous and/or artificial promoter.

11. The polynucleotide construct of any of claims 6-10, wherein the polynucleotide is DNA.

12. The polynucleotide construct of any of claims 6-11, wherein the construct is extrachromosomal and comprises one or more sequence(s) providing autonomous replication and/or autonomous maintenance in a host cell.

5 13. The polynucleotide construct of any of claims 6-12, which is integrated into the genome of a host cell.

14. The polynucleotide construct of any of claims 6-13, which is a plasmid, a linearized plasmid, or a multimerized plasmid.

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15. The polynucleotide construct of claim 14, wherein the plasmid comprises at least one origin of replication that is functional in a host cell.

15 16. The polynucleotide construct of any of claims 6-15, which further comprises at least one selection marker gene which encodes a polypeptide which in turn confers resistance to an antibiotic when expressed in a host cell.

17. A cell comprising at least one exogenous marker gene encoding an orotate transporter polypeptide comprising an amino acid sequence at least 60% identical to SEQ ID
20 NO: 2.

18. The cell of claim 17, wherein the at least one marker gene is a selection marker, a screening marker, a counter-selection marker, or a bi-directional selection marker.

25 19. The cell of claim 17 or 18, wherein the at least one marker gene encoded orotate transporter polypeptide also transports one or more orotate analogues.

20. The cell of any of claims 17-19, wherein the at least one marker gene encoded orotate transporter polypeptide also transports the orotate analogue 5-fluoroorotate (FOA).

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21. The cell of any of claims 17-20, wherein the at least one marker gene is transcribed from at least one heterologous and/or artificial promoter.

22. The cell of any of claims 17-21, which is a microbial cell.

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23. The cell of any of claims 17-21, which is a bacterial cell.

24. The cell of any of claims 17-21, which is a Gram-negative or Gram-positive bacterial cell.

25. The cell of any of claims 17-21, which is of the genus *Lactobacillus*, *Bacillus*, or *Escherichia*.

26. A method of selecting or identifying a cell comprising at least one copy of a recombinant marker gene, and/or selecting or identifying a cell which has been cured of the recombinant marker gene, wherein said marker gene encodes an orotate transporter polypeptide comprising an amino acid sequence at least 60% identical to SEQ ID NO: 2, said method comprising the step of using the marker gene as a selection marker, a screening marker, a counter-selection marker, or a bi-directional marker, under suitable conditions, whereby the cell is selected or identified.

27. The method of claim 26, wherein the cell is pyrimidine auxotrophic and lacks a functional orotate transporter protein in the absence of the recombinant marker, and wherein the recombinant marker is introduced into the auxotrophic host cell, which is then cultivated in a growth medium with no uracil but supplemented with orotate, wherein only the cell comprising the recombinant marker will grow, wherein the marker is used as a selection marker.

28. The method of claim 26, wherein the cell is pyrimidine auxotrophic and comprises the recombinant marker gene which encodes a functional orotate transporter protein, and wherein the marker gene is then cured from the cell, which is cultivated in a growth medium with no uracil, wherein only the cell cured of the marker gene is inhibited, wherein the marker is used as a screening marker.

29. The method of claim 27 or 28, wherein the cell is pyrimidine auxotrophic due to a mutation in at least one gene encoding an enzyme which converts dihydro-orotate to orotate.

30. The method of claim 29, wherein the cell is pyrimidine auxotrophic due to a mutation in one or more of *pyrD*, *pyrDa*, *pyrDb*, and *pyrK*.

31. The method of claim 26, wherein the cell lacks a functional orotate transporter protein in the absence of the recombinant marker, and is resistant to the orotate analogue 5-fluoroorotate (FOA), and wherein the recombinant marker is introduced into the cell, which is then cultivated in a growth medium supplemented with an inhibitory concentration of FOA,

wherein only the cell comprising the recombinant marker is sensitive to FOA and is inhibited, wherein the marker is used as a screening marker.

32. The method of claim 26, wherein the cell comprises the recombinant marker gene and is sensitive to 5-fluoroorotate (FOA), and wherein the marker gene is then cured from the cell, which is cultivated in a growth medium supplemented with an inhibitory concentration of FOA, wherein only the FOA-resistant cell cured of the recombinant marker gene will grow, wherein the marker is used as a counter-selection marker.

33. The method of claim 26, wherein the cell comprising at least one copy of the recombinant marker gene is first selected or identified, and subsequently a cell which has been cured of the recombinant marker gene is selected or identified, wherein the marker is used as a bi-directional marker.

34. The method of claim 33, wherein the cell is resistant to 5-fluoroorotate (FOA), pyrimidine auxotrophic, and lacks a functional orotate transporter protein in the absence of the recombinant marker, and wherein the recombinant marker is first introduced into the orotate auxotrophic host cell, which is then cultivated in a growth medium supplemented with orotate, wherein only the cell comprising the recombinant marker will grow, and subsequently the marker gene is then cured from the cell by cultivation in a growth medium supplemented with an inhibitory concentration of FOA, wherein only the FOA-resistant cell cured of the recombinant marker gene will grow, wherein the marker is used as a bi-directional selection marker.

35. The method of claim 34, wherein the cell is pyrimidine auxotrophic due to a mutation in a gene encoding an enzyme which converts dihydro-orotate to orotate.

36. The method of claim 34, wherein the cell is pyrimidine auxotrophic due to a mutation in one or more of *pyrD*, *pyrDa*, *pyrDb*, and *pyrK*.